

FIGURE 1: Construct of Feline Thyrotropin beta-subunit with First Intron

1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu
 31 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
 76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu
 121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr ile cys ala gly tyr cys met thr arg
Intron 1
 163 581 625
 GTATGTAGTTCATCTCACTTCTTTAGCTGAAAATTAGATAAACCTAGACT
 CAGTCCATTCTATCCAGAAAGGAAATGAGATAAATCACACCTCATTACAG
 ACCTAACGGTCATTGGCTCCTTAGAGGGTAGAGTCCCTAGGGTATAATACCGA
 CCTACTCCATACAGTTGGTACAGATAATTTACAATAGTTTACTCCCAAAGTT
 TATTTAAACCTTATCTTGTCCCACGATCAAGGATAAAAGAGAGGGTGTGTGT
 ATGTCATTTTTTTGTCTATAGGATTCAAGTGTGGATATGCTGAATTGGTATT
 GGGGAATGGGACTAAGGAATCCTCCCCAGTCCTATTGTATCTATGGGATGT
 AAGCGAATTAACATTTGCTCCTCTGTGCTCCCTCAG 580
 626 670 715
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
 626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
 671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val
 716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
 761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
 806 835
 AAG TCC GAT GTG GTA GGA GTT TCT ATC TAA (GCGGCCGC₍₄₎)(AT)₅-3'
 lys ser asp val val gly val ser ile stop

() denotes the Eco RI restriction sites

Bold denotes signal sequence

Bold/italic denotes the intron 1 sequence

FIGURE 2: Feline Thyrotropin alpha-subunit Construct

(GAATTC) GCCCTT

1

45

AGT TAC TGA GAA ATC ACA AGA CGA AGC CAA AAT CCC TCT TCA GAT

46

90

CCA CGG TCA ACT GCC CTG ATC ACA TCC TGC AAA AAG TCC GGA GGA

91

135

AGG AGA GCC ATG GAT TAC TAC AGA AAA TAT GCA GCT GTC ATT CTG

met asp tyr tyr arg lys tyr ala ala val ile leu

136

180

GCC ATA CTC TCT GTG TTT CTG CAT ATT CTC CAT TCT TTT CCT GAT

ala ile leu ser val phe leu his ile leu his ser phe pro asp

181

225

GGA GAG TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA

gly glu phe thr met gln gly cys pro glu cys lys leu lys glu

226

270

AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG

Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met

271

315

GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG

gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys

316

360

AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC

lys thr met leu val pro lys asn ile thr ser glu ala thr cys

361

405

TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC

cys val ala lys ala phe thr lys ala thr val met gly asn ala

Continued on next page

Bold denotes 24 amino acid signal sequence as per structure in other species

Bold italics denotes sequence upstream from expressed but not secreted signal sequence that results in enhanced expression of the construct.

() denotes Eco R1 restriction site from TOPO Blunt vector

Underlined denotes additional sequence from TOPO Blunt vector

(1) denotes Factor XA site

(2) denotes Flag tag

(3) denotes stop codon

(4) denotes Not1 restriction enzyme site

()⁵ denotes extra bases needed for restriction enzyme to work

Figure 2 cont.

FIGURE 3: Yoked Feline Thyrotropin

FIGURE 3. *Plasmodium yoelii*
 1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu
 31
 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
 76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu
 121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg
Intron 1
 163 GTATGTAGTTCATCTCACCTCTTAGCTGAAAATTAGATAAACCTAGACT
 CAGTCCATTCTATCCAGAAAGGAAATGAGATAAATCACACCTCATTACAG
 ACCTAACGGTCATTGGCTCCTTAGAGGGTAGAGTCCCTAGGTTATAATATACGGA
 CCTACTCCATACAGTTGGTACAGATAATTTACAATAGTTTACTCCCAAAGTT
 TATTTAACCTTATCTTGTCTCCACGATCAAGGATAAAAGAGAGGGTGTGTGT
 ATGTCATTTTTTTGTCTATAGGATTCAAGTGTGGATATGCTGAATTGGTATT
 GGGGAATGGGACTAAGGAATCCTCCCCAGTCCTATTGTATCTATGGGATGT
 AAGCGAATTAACATTTGCTCCTCTGTGCTTCCCTCAG 580
 581 625
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
 626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
 671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val
 716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
 761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
 806 *beta-specific primer sequence* ~~CTP linker~~ 850
 AAG TCC GAT GTG GTA GGA GTT TCT ATC CAG GAC TCC TCT TCC TCA
 lys ser asp val val gly val ser ile gln asp ser ser ser ser
 851 *CTP linker* 892
 AAG GCC CCT TCC GCC AGC CTT CCA AGC CCA ACG CGT CTC CCG
 lys ala pro ser ala ser leu pro ser pro thr arg leu pro

*reverse complement in construct

AfI III ligation site

*alpha-specific
primer sequence*

893	<i>CTP linker</i>	937	*
GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA		TTT CCT GAT GGA GAG	
gly	pro ser	asp thr	gly
938		pro ile	ile
		pro gln	phe
		phe pro	asp gly
		asp glu	glu
977			
TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA			
978			
phe thr met gln gly cys pro glu cys lys leu lys glu			
1022			
AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG			
Asn	lys	tyr	phe ser
1023	lys	leu	gly ala
	ala	pro	ile tyr
		gln	cys met
1067			
GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG			
gly	cys	cys phe ser arg	ala tyr pro thr pro ala arg ser lys
1068			
1112			
AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC			
lys	thr met leu val pro lys	asn ile	thr ser glu ala
1113	lys	ala	thr cys
1157			
TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC			
cys	val	ala lys	ala phe thr lys
1158	ala	ala	ala thr val
1202			met gly asn ala
AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC			
lys	val	glu asn his	thr glu cys his cys ser
1203	lys	his	thr cys tyr his
1211			
CAC AAG ATT (ATC GAA GGT CGT₍₁₎)(GAC TAC AAG GAC GAT GAC GAT			
his	lys	ile	ile glu gly arg
1245	1247		asp tyr lys
			asp asp asp asp
1262			
AAG₍₂₎ (TAA₍₃₎) (GC GGCCGC₍₄₎)(TATG)₅ 3'			
lys			

* as written

Figure 3 cont.

KEY

() denotes the Eco RI restriction sites

Bold denotes signal sequence***Bold italics*** denotes intron 1 sequence 1=Factor XA site

((1)) denotes Factor XA site

((2)) denotes Flag tag

((3)) denotes stop codon

((4)) denotes Not1 restriction enzyme site

()₅ denotes extra bases needed for restriction enzyme to work

Figure 3 cont.

PEAK Expression Vector (with yoked fTSH)

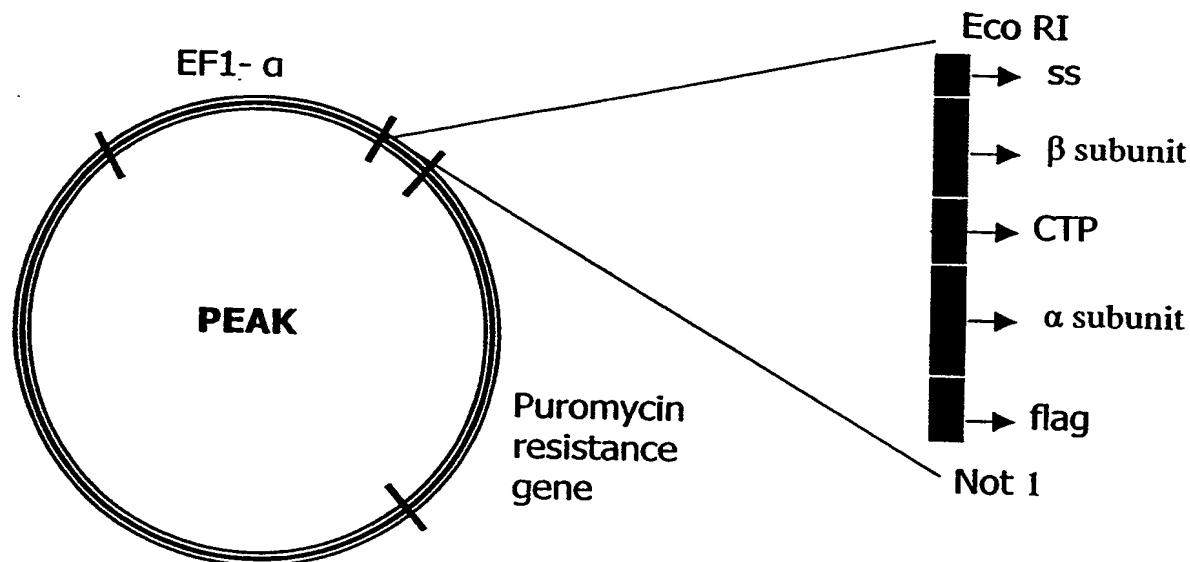


Figure 4



Figure 5a

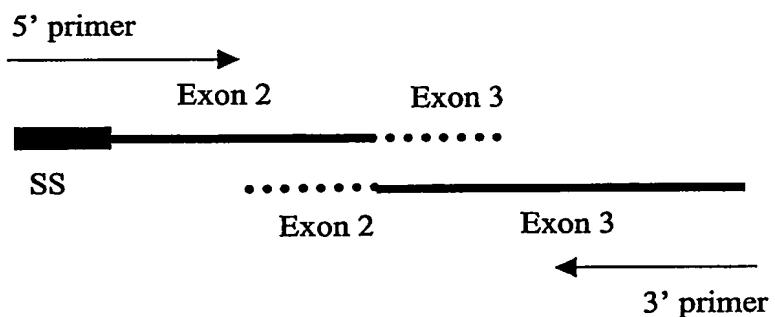


Figure 5b

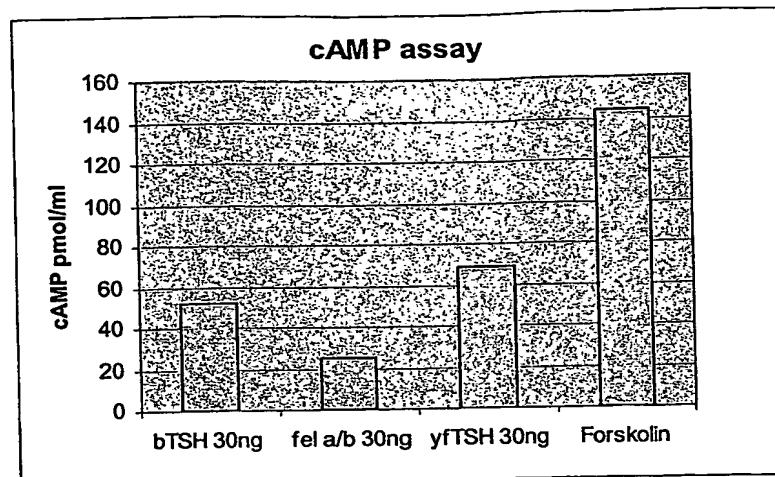


Figure 6a

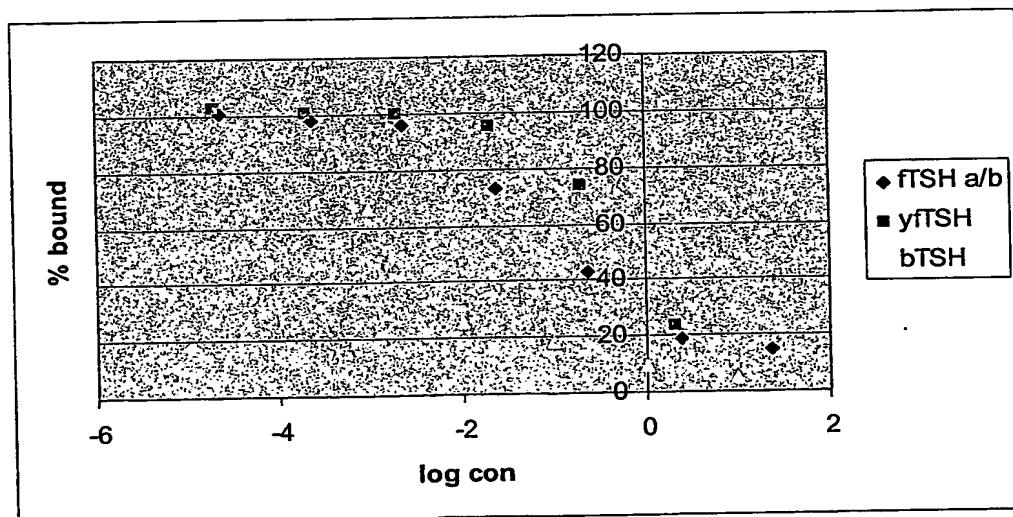


Figure 6b